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REVIEW ARTICLE

Coagulopathy during COVID-19 infection: a brief review

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Abstract

The COVID-19 pandemic caused by SARS-CoV-2 continues to spread rapidly due to its virulence and ability to be transmitted by asymptomatic infected persons. If they are present, the symptoms of COVID-19 may include rhinorrhea (runny nose), headache, cough, and fever. Up to 5% of affected persons may experience more severe COVID-19 illness, including severe coagulopathy, acute respiratory distress syndrome (ARDS) characterized by respiratory failure that requires supplementary oxygen and mechanical ventilation, and multi-organ failure. Interestingly, clinical evidence has highlighted the distinction between COVID-19-associated coagulopathy (CAC) and disseminated intravascular coagulation (DIC). Patients with CAC exhibit different laboratory values than DIC patients for activated partial thromboplastin time (aPTT) and prothrombin time (PT) which may be normal or shortened, varying platelet counts, altered red blood cell morphology, unique bleeding complications, a lack of schistocytes in the peripheral blood, and no decrease in fibrinogen levels. In this review, we consider the search for 1) laboratory results that can diagnose or predict development of CAC, including serum levels of D-dimers, fibrinogen, interleukin-6 (IL-6) and the growth factor angiopoietin-2 (Ang-2), 2) mechanisms of CAC induction, and 3) novel therapeutic regimens that will successfully treat COVID-19 before development of CAC.

Keywords COVID-19 · SARS-CoV-2 · Coagulopathy · COVID-19-associated coagulopathy (CAC)

Introduction

Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is a member of the genus Betacoronavirus, subgenus Sarbecovirus. SARS-CoV 2 shares significant homology with the virus that causes SARS, SARS-CoV1 [1–3]. While the original source of SARS-CoV-2 has not been identified, similar strains have been found in bats and Malayan pangolins [1–3].

Within months of being identified (in March 2020), COVID-19 had reached global pandemic levels, triggering lockdowns across nations to hinder its spread. The virus has continued to spread rapidly through human populations due to its virulence and ability to be spread by infected persons who are asymptomatic. In addition to typical symptoms of upper respiratory illnesses including rhinorrhea (runny nose), fever, headache, and cough, COVID-19 can result in more severe illness in up to 5% of those affected. One of its most severe complications is acute respiratory distress syndrome (ARDS), leading to many hospitalized patients requiring ventilators to provide sufficient oxygen for the patients to survive. Unfortunately, despite the existence of several effective medical interventions that are described herein and a preventive vaccine, over 5.4 million people across the world have died from COVID-19 as of January 2022, according to the World Health Organization (WHO) [4]. With the current total of confirmed cases at almost 282 million globally, this represents a global death rate of 1.9%. In the USA, there have been over 52.5 million confirmed cases and over 812,000 deaths equating to a national death rate of 1.5% [4]. Although by early January 2022, almost 8.7 billion vaccine doses had been administered worldwide, including over 485 million doses administered in the USA [4], the vaccination of less developed nations lagged behind that in the rest of the world. With a global population of approximately 7.9 billion and 1–3 doses (or more for immunocompromised individuals depending on the vaccine) being

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required for an individual to be considered fully immunized, global vaccination remains a vast and urgent international priority [5, 6].

During the pandemic, hospital personnel have had to wear many layers of uncomfortable personal protective equipment (PPE), take extra precautions when treating any patient whose COVID-19 status is unknown, work overtime to cover shifts of coworkers who become ill, resign, or quarantine, and endure the illness themselves should they become infected. It has proven incredibly difficult to understand and treat the myriad of symptoms and syndromes associated with COVID-19, particularly in understaffed hospitals with overworked employees. While treating and observing patients with COVID-19, health providers and laboratorians sought laboratory values that would predict disease severity and mortality, thereby facilitating more proactive treatment of the sickest patients. It soon became clear that COVID-19 causes not only ARDS and respiratory failure but can lead to very significant coagulopathy and multi-organ failure. At the beginning of the pandemic, the most informative research on COVID-19-associated coagulopathy (CAC) was derived from autopsies of patients who had died from CAC. The abnormal laboratory values from coagulation studies were found to represent massive microvascular clotting in the lungs and, in some cases, even in other organs. Since this discovery, many investigators have sought to determine the mechanism leading to CAC, as well as how to properly treat and/or prevent it.

CAC has added another level of severity to an already complex disease. Most hospitalized COVID-19 patients already present with hypoxia and substantial respiratory distress, so the addition of a potentially fatal coagulopathy further presses hospital staff to monitor laboratory coagulation values and creates the need for additional medications to be administered and monitored. CAC continues to overwhelm healthcare workers and cause increased mortality. Defining and understanding the complex mechanisms of CAC is of vital importance so that preventive measures can be implemented to prevent further loss of life associated with CAC.

Laboratory values as predictors of CAC

Since the onset of the pandemic, investigators have continued to seek laboratory values that can aid in predicting the severity of disease and risk of mortality in hospitalized COVID-19 patients. Several laboratory values have been identified that correlate with disease severity and are indicative of the hypercoagulable state exhibited by most critical COVID-19 patients. Some of these include elevated D-dimer levels, which serve as markers for both clotting and fibrinolysis [7]; decreased levels of procoagulant proteins including factor V, factor VIII, and fibrinogen [8]; and increased levels of inflammatory markers (acute phase reactants) including α1-acid glycoprotein, C-reactive protein (CRP), ferritin, procalcitonin, and high-sensitivity troponin [8, 9]. However, while these markers are sensitive indicators of disease severity, they are relatively non-specific and their relationship to CAC warrants more evaluation. Other helpful markers include altered levels of the cytokine interleukin-6 (IL-6) [10] and the inflammatory marker angiopoietin-2 (Ang-2), released after endothelial cell damage [11]. Each of these factors are considered in detail below.

Markers that are less helpful in predicting the severity of and outcomes for COVID-19 infection include platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), serum levels of anticoagulant proteins such as heparin, lupus anticoagulant (LAC), and IL-6-regulated proteins C, S, and antithrombin (AT), and anti-phospholipid antibodies (aPL) [12, 13]. While these markers may help to understand CAC pathophysiology, they have not proven useful in the diagnosis or treatment of COVID-19.

D-dimers

The laboratory value that serves as the best predictor of disease severity and mortality is the level of D-dimers in serum. When the body forms a stable thrombus (blood clot) comprised of fibrin and stabilized by factor XIII [14], the clot is subsequently broken down by the fibrinolytic enzyme plasmin to form D-dimers, among other products. Since the level of D-dimers in patient serum can monitor both thrombus formation and fibrinolysis, elevated D-dimer levels indicate the presence of excessive clot formation and breakdown. In almost every patient hospitalized with COVID-19, the finding of elevated D-dimer levels reflects hypercoagulability and marked elevation of fibrinolysis [7]. The D-dimer level is the most consistently validated laboratory value that directly predicts disease severity related to the patient's coagulation status. While it is a sensitive and specific marker, the D-dimer level cannot identify the precise area of the body where the clotting and fibrinolysis is occurring [15]. D-dimer levels are non-specific for determining whether a patient is experiencing microvascular clotting in the lungs or venous thromboembolisms in the legs but consistently provides the best value for monitoring the presence of clotting, the amount of clotting and fibrinolysis occurring in a patient, and the efficacy of antithrombotic treatment.

Interestingly, despite its widespread use, there are some caveats to its utility for this purpose. In the absence of functional fibrinolysis by plasmin, D-dimer levels will neither be elevated nor accurately portray the extent of clotting that is occurring in the body even if a patient is in a hypercoagulable state. This is also true in patients with elevated D-dimer levels exhibiting impaired fibrinolysis. A small-scale study assessed tissue plasminogen activator-induced fibrinolysis in five patients hospitalized with

severe COVID-19 and five healthy controls using thromboelastometry [16]. The results showed that the patients exhibited impaired fibrinolysis as the result of hyperfibrinogenemia, resulting in decreased D-dimer levels [16]. However, this study also suggested that treatment with recombinant tissue plasminogen activator might be useful, although the effects could be transient [17]. Another study found that maximum lysis of fibrin clots was decreased in patients with severe COVID-19 disease and resistance to fibrinolysis was strongly linked to disease severity [18]. If maximum fibrinolysis is reduced in severe COVID-19 patients, healthcare providers must take this into consideration when assessing the severity of a patient's CAC using D-dimer as an indicator.

Fibrinogen

The hypercoagulability marker fibrinogen has also been extensively evaluated for its utility in predicting the severity of COVID-19 disease. Fibrinogen is markedly elevated in critically ill COVID-19 patients and is correlated with higher mortality due to its ability to promote coagulation [9, 14–21]. For example, one study reported that 90% of patients with intermediate to critical COVID-19 exhibited fibrinogen levels above the reference range [18, 22]. These results are consistent with reports that SARS-CoV-1 infection caused upregulation of fibrinogen expression in infected cells and was associated with microvascular clots in the lungs of SARS patients [23, 24]. Due to these findings, D-dimer and fibrinogen levels are often monitored together to form a picture of a patient's coagulation status.

Interleukin-6 (IL-6)

The cytokine IL-6 is an important player in coagulation as it downregulates clotting inhibitors protein C, protein S, and antithrombin (AT) and is also the key pathogenic cytokine involved in instigating a cytokine storm. The use of IL-6 inhibitors as part of an early COVID-19 treatment regimen, particularly for those with ARDS, was proposed to combat significantly elevated levels of the cytokine. Observations revealed that IL-6 levels were above the reference range in both COVID-19 survivors and non-survivors, but the levels in non-survivors were astoundingly higher than those of survivors [10]. This suggests that patients with severe COVID-19 who are experiencing a hyperimmune response and who exhibit extremely high levels of IL-6 are more likely to experience CAC associated with mortality [10]. Many studies maintain that serum IL-6 is the best available biomarker to indicate immune system dysregulation and the likelihood of severe COVID-19 disease culminating in death [10, 25, 26].

Angiopoietin-2 (Ang-2)

The growth factor angiopoietin-2 (Ang-2) plays both agonistic and antagonistic roles during angiogenesis, depending on the cellular context. Under normal conditions, levels of Ang-2 are low, but its expression in endothelial cells can be upregulated by inflammatory conditions including cancer and hypoxia, and by inflammatory mediators such as thrombin [11]. Although Ang-2 is stored within endothelial cells, it can be released following cellular damage, for example, on entry of SARS-CoV-2 or after apoptosis. Under these conditions, Ang-2 increases endothelial cell permeability, causes detachment of pericytes from the basement membrane, and permits migration of immune or cancer cells across the endothelial barrier. Historically, Ang-2 levels have directly correlated with the severity of coagulation disorders in patients with sepsis as it is a direct assessment of endothelial cell damage. This is also true for COVID-19 patients, as the level of Ang-2 is more sensitive and specific than D-dimer levels and is thus a better predictor of vascular damage leading to hypercoagulability [20].

Platelet count

Platelet counts have proven to be unreliable as outcome predictors of COVID-19 infection. Despite the numerous and widespread thrombi found on autopsy of deceased patients, COVID-19 patients do not exhibit significant levels of clinically relevant thrombocytopenia for reasons that remain unclear [9, 27]. In fact, platelet counts remain normal or only decrease slightly despite extensive CAC. Wool and Miller described an association between COVID-19 and elevated numbers of immature platelets, despite normal overall platelet counts [27]. Increases in immature platelet numbers is indicative of increased platelet storage pool release in the bone marrow or an increased rate of release, which could explain the maintenance of a normal platelet count despite massive clotting. Because they are more functional and reactive, it is possible that increased levels of immature platelets could contribute to hypercoagulation in patients with severe COVID-19 infection. There is also evidence for extramedullary platelet production by megakaryocytes in COVID-19 patients that could also account for the rarity of thrombocytopenia in these patients [15]. These results suggest that compensatory mechanisms that can stabilize platelet counts amid severe microvascular coagulation likely occur during COVID-19 infection, making them an unreliable marker for predicting coagulopathy or disease severity.

CAC versus disseminated intravascular coagulation (DIC)

Analysis of platelet counts in COVID-19 patients has clarified that a distinction exists between CAC and disseminated

intravascular coagulation (DIC; also known as consumption coagulopathy). Early in the pandemic, investigators described the hypercoagulation they observed in these patients as DIC. However, a distinction between DIC and CAC was identified based on coagulation data and bleeding occurrences in COVID-19 patients. Noticeably disparate laboratory values were observed in activated partial thromboplastin time (aPTT), prothrombin time (PT), and platelet counts. In CAC, the PT may be within the reference range or shortened but not prolonged, and there is no decrease in fibrinogen levels. Additionally, red blood cell morphology and bleeding complications also differed in that CAC exhibited a distinctive lack of schistocytes in the peripheral blood, while these peripheral blood schistocytes are commonly observed in patients with DIC [25].

Identifying these clinical differences between CAC and DIC is critically important because many strategies applied in the treatment of DIC cannot be used to treat patients with CAC, such as the administration of concentrated clotting factors or fibrinogen which is unnecessary and could, in fact, lead to adverse effects. Unlike DIC, CAC is not characterized by increased activation of pre-coagulant pathways and results in only mild thrombocytopenia in even the most critically ill patients. Iba and colleagues (2020) indicated that in their study fewer than 7% of COVID-19 patients with coagulopathy met the International Society on Thrombosis and Hemostasis diagnostic criteria for DIC [28, 29]. The pattern of coagulopathy in conjunction with relatively normal PT, aPTT, and antithrombin values observed in patients with CAC is also dissimilar to the typical pattern seen in sepsis-induced coagulopathy (SIC) [28]. Based on these observations, a collection of laboratory findings that includes increased D-dimer levels, elevated levels of fibrinogen and von Willebrand factor (VWF), and relatively normal PT, aPTT, and platelet count were proposed to diagnostic and prognostic indicators of CAC [20, 28].

Platelet induction of neutrophil extracellular traps (NETs)

Platelet function plays a significant role in the pathogenesis of COVID-19, significantly impacting the course of the disease. Typically predictive of increased mortality, immunothromboses, microvascular clots, and macrovascular clots including deep vein thromboses (DVT) and pulmonary emboli (PE) have been found in COVID-19 patients [30]. Several hypotheses have been proposed to account for platelet contributions to CAC. Engelmann and Massberg (2013) [31] coined the term "immunothrombosis" to describe the symbiotic relationship that exists between the innate immune and coagulation systems that facilitate recognition of damaged cells or pathogens and initiate the cellular and molecular interactions that culminate in the formation of a thrombus [25, 31]. Investigators have speculated that the formation of tiny platelet- and neutrophil-rich immunothrombi in the pulmonary blood vessels can effectively plug the vessel to prevent further movement of viruses into lung tissue [18]. This hypothesis would be consistent with the clots comprised of platelets and IgG immune complexes that are formed in the lung microcirculation at the beginning of a COVID-19 infection and may prevent further SARS-CoV-2 invasion into lung tissue [25, 32].

The interaction that occurs between the coagulatory and immune responses is very sensitive and must be carefully regulated to prevent a downstream cascade of immune-mediated thrombus formation [25]. For example, immunohistochemical staining of lung tissue obtained by autopsy from COVID-19 non-survivors revealed substantial deposition of microimmunothrombi in the microvasculature of the lungs [18]. The presence of these circulating platelet-neutrophil aggregates was directly and positively correlated with the severity of COVID-19 pulmonary disease and respiratory damage, indicating that platelets play a role in activation of neutrophils and boost neutrophil-driven tissue damage [18, 25].

Activation of platelets by antibody-antigen complexes induces a procoagulant response that results in platelets binding to the endothelium, releasing soluble coagulation factors, and uncovering negatively charged phospholipids that serve as cofactors for the proteolytic reactions accomplished by the coagulation factors [25]. Activated platelets attract neutrophils and release neutrophil extracellular traps (NETs) consisting of DNA, histones, and other antimicrobial contents in the presence of pathogen-associated molecular patterns (PAMPs) and cytokines, a process called NETosis [25, 33, 34]. NETosis can be considered a procoagulant process, as NETs activate both the intrinsic and extrinsic coagulation pathways by exposing tissue factor and activating factor XII in addition to activating and aggregating platelets [25, 35]. Autopsy-derived histopathology studies on deceased COVID-19 patients demonstrate the common findings of NETs and platelet aggregates, suggesting another possible mechanism of hypercoagulation in COVID-19 patients [18, 35]. Consistent with these findings, serum markers of neutrophil activation and NETosis are elevated in patients with severe COVID-19 [35].

Endotheliopathy and coagulopathy

The endotheliitis associated with COVID-19 provides another possible mechanism for development of CAC. Binding of SARS-CoV-2 to its cell surface receptor, angiotensin-converting enzyme 2 (ACE2), on vascular endothelial cells and the virus's subsequent entry into the cell via fusion between the viral lipid envelope and the plasma membrane causes sufficient cellular damage that procoagulant factors

are released, and the usual antithrombotic activity of the vascular lumen is inhibited [20]. Healthy endothelial cells synthesize nitric oxide synthase that converts L-arginine to L-citrulline, leading to the production of nitric oxide (NO). Release of NO prevents adhesion of platelets and leukocytes, migration of immune cells into the vessel wall, proliferation of smooth muscle, and suppression of inflammation and apoptosis. The loss of normal ACE2 activity leads to reduced ACE2-mediated inhibition of angiotensin II and its reduced conversion to angiotensin. Accumulation of active angiotensin II stimulates vascular constriction. The reduction in angiotensin levels leads to a decrease in NO, resulting in a hypercoagulable state that, together with vasoconstriction and adhesion of platelets and leukocytes, increases thrombus formation [20].

Injury to these vascular endothelial cells during entry of SARS-CoV-2 complexed with ACE-2 also stimulates the innate immune system to produce several proinflammatory cytokines, including IL-6 as described above [35]. The simultaneous production of these cytokines in thousands of vascular endothelial cells can result in a cytokine storm in patients with severe COVID-19. The endothelial injury and hyperimmune response are directly involved in the subsequent development of the hypercoagulable state observed in patients with CAC [10].

A study of gene expression in peripheral blood mononuclear cells (neutrophils) and lung endothelial cells obtained by bronchoalveolar lavage from healthy controls and SARS-CoV-2-positive individuals provides a new perspective on genetic changes induced by the virus in pulmonary endothelial cells that could make the body more susceptible to hypercoagulation [19]. The most significant findings of this in vitro study were the direct activation by SARS-CoV-2 of the extrinsic coagulation pathway and its downregulation of the plasminogen activation system. Thus, the virus itself promotes coagulation and hinders the anticoagulative response by inhibiting activation of plasminogen to plasmin, thereby preventing dissolution of fibrin clots [19].

Macrovascular thrombosis

COVID-19 patients exhibit not only extensive microvascular clotting detectable only on autopsy, but also macrovascular including DVT and PE even after thromboprophylactic treatment [27]. In a review of several clinical studies, Wool and Miller calculated the frequency of thrombotic complications to be significantly variable between 16 and 69% in COVID-19 patients admitted to intensive care units (ICUs). The higher percentage was observed in a study utilizing ultrasonography to screen hospitalized COVID-19 patients for DVT [27]. A concerning factor remains the observation that despite thromboprophylaxis, venous thromboembolisms (VTEs) are still found at elevated levels in both survivors and non-survivors. In one large-scale study, 31% of patients still exhibited VTEs despite daily administration of Nadroparin thromboprophylaxis (2850–5700 IU, based on body weight) [15]. These findings indicate that significant questions remain regarding the type and dosage of thromboprophylaxis that will be needed to prevent thromboses in COVID-19 patients.

The contribution of extracorporeal membrane oxygenation (ECMO) to CAC

ECMO can be used to preserve life in patients with severe COVID-19 who have lost adequate lung and heart function due to CAC and other pathological effects [36]. The external ECMO machine functions as a pump capable of removing carbon dioxide and returning oxygenated blood through cannulas placed in several of the patient's arteries and veins [36]. Used as a last resort in COVID-19 patients, the ECMO system can promote further hypercoagulation once the coagulation cascade has been activated. When thrombin is generated in the coagulation cascade, fibrin can be deposited on the surfaces of the ECMO machine, possibly contributing to a total dysregulation of the coagulation system [37]. The initiation of extracorporeal support also initiates an inflammatory response, leading to upregulation of prothrombotic pathways and, to a lesser extent, the fibrinolytic pathways [37]. Yet this coagulatory state is sufficiently dynamic that patients on ECMO can switch quickly from a procoagulant state, causing venous thromboembolisms, to an anticoagulant state, leading to excessive bleeding associated with increased fibrinolysis, decreased platelet adhesion, and consumption of coagulation factors [37]. Due to this, patients on ECMO require constant monitoring and testing of laboratory values including D-dimer and fibrinogen, with extra attention paid to changes in aPTT and PT, as these may be the only values that demonstrate a shift as the patient moves from one state to the other.

Treatment and prevention of CAC

Physiologic anticoagulants

Many COVID-19 patients experience thrombotic events despite prophylactic administration of anticoagulants. Still, physiologic anticoagulants such as protein C and antithrombin are being studied to determine their potential utility in preventing coagulation and reducing inflammation that can lead to lung injury [20]. Recombinant activated protein C was evaluated in a large-scale study in patients with DIC and sepsis but was discontinued because its major side effect was bleeding [38]. Although traditional DIC does not present in COVID-19 patients, this side effect should be carefully considered if trials move forward using recombinant

activated protein C as a treatment for CAC, especially given the predisposition of these patients to quickly shift coagulation states.

Heparin

The use of heparin as a prophylactic and therapeutic treatment for both macrovascular and microvascular clotting has been widely discussed and disputed in the literature, primarily due to disagreement pertaining to its effectiveness in the prevention and treatment of thrombus formation. However, healthcare providers generally consider it to be useful to administer prophylactic doses of low molecular weight heparin (LMWH) or unfractionated heparin upon admission of a patient to the hospital, regardless of whether coagulation laboratory values are elevated [21, 28]. A survey of hospital protocols for treatment of COVID-19 and related complications revealed that the area of greatest agreement in protocol was the use of heparin-based pharmacologic prophylaxis against formation of venous thromboembolism (VTE) [21]. In the case of confirmed VTEs, most of the reviewed protocols suggest that patients should receive a calculated dose of LMWH or unfractionated heparin, based on weight and renal condition, for at least 3 months [21]. In light of the additional anti-inflammatory benefits of heparin administration and given the hypercoagulable state that causes death in COVID-19 patients, Bhattacharjee et al. (2020) [38] advised that it is critical to administer therapeutic unfractionated heparin or LMWH to prevent the formation of microvascular and macrovascular thromboses [38]. It is worth reiterating that the administration of an anticoagulant to a patient who is experiencing a delicate coagulopathy should be monitored closely, since the optimal dosage is not known, and bleeding can occur [39].

Tocilizumab

Tocilizumab (marketed by Genentech as Actemra) is a monoclonal antibody therapy directed against human cytokine IL-6, a major initiator of hypercoagulability and the cytokine storm in COVID-19 patients, as noted above [40–43]. The FDA issued an emergency use authorization (EUA) in June 2021 for use of tocilizumab to treat inpatients (adults and children over 2-years-old) receiving systemic corticosteroids, supplemental oxygen, and either mechanical ventilation or ECMO [44]. Early studies of tocilizumab were hampered by the clinical trials being uncontrolled, non-randomized studies performed in patients also receiving other treatments. In a clinical trial performed in China, 100% of patients ($n=21$) with severe COVID-19 infection and some on ventilators recovered after being administered a single dose of intravenous tocilizumab [41]. These patients also experienced a dramatic decrease in the levels of inflammatory cytokines and symptoms and a swift increase in blood oxygen levels [41]. Importantly however, these patients were also being concurrently administered supplemental oxygen, corticosteroids (including methylprednisolone), antiviral treatments (lopinavir), and empirical treatment for symptom relief [41], making it difficult to attribute the improvement solely to any one of these treatments.

In another tocilizumab trial conducted in Qatar ($n=25$), of the 84% of patients who began the trial on ventilators, only 28% remained ventilated after 14 days of administration of tocilizumab and only 12% died [43]. Additionally, C-reactive protein was continually monitored and was remarkedly decreased seven days after tocilizumab was administered [42, 43]. However, because these patients were also receiving other treatments including interferon-α, antiviral lopinavir/ritonavir, azathioprine (an immune suppressant used to prevent rejection of transplanted kidneys), and hydroxychloroquine (an antimalarial agent), determining the effects of tocilizumab alone from this regimen remains difficult.

The data that support the EUA issued by the U.S. FDA for tocilizumab were generated through four clinical trials: RECOVERY, EMPACTA, COVACTA, AND REMDACTA, with greater emphasis on the results of the RECOVERY and EMPACTA trials [40, 45–48]. One randomized, placebo-controlled, double-blind trial of unventilated, hospitalized COVID-19 patients ($n=389$) revealed that following 28 days of treatment with tocilizumab, 12% of the tocilizumab group ($n=249$) required mechanical ventilation and/or died, compared to 19.3% for the placebo group. Death from any cause by day 28 was 8.6% for the placebo group and 10.4% for the tocilizumab group. These data reveal that while tocilizumab was to a limited extent able to reduce progression to mechanical ventilation or death, it failed to improve overall survival [45]. In the Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial, a total of 4,116 hospitalized adults with COVID-19 that were experiencing hypoxia and evidence of systemic inflammation classified by high levels of CRP were used to assess the effectiveness of treatment with tocilizumab. Of the total number of patients, 3,385 were also receiving systemic corticosteroids [40]. The control group received the usual standard of care, whereas the tocilizumab group received the usual standard of care plus an intravenous 400–800 mg dose (dependent on weight) of tocilizumab. If the patient's condition had not improved within 12–24 h, a second dose was administered [40]. Within 28 days, 31% of patients receiving tocilizumab (621/2,022) and 35% (729/2,094) of patients in the control group expired. Data indicated that patients in the tocilizumab group were more likely to be released from the hospital within 28 days. Of the patients who were not receiving mechanical ventilation at the start of the trial, those treated with tocilizumab were less likely to require mechanical ventilation and/or die [40]. Thus, for hospitalized COVID-19 patients, the addition of

tocilizumab to the care regimen reduced the risk of being placed on a ventilator or dying through 28 days of treatment and decreased the time of hospitalization [40, 44, 45].

Antiplatelet drugs

Prophylactic administration of aspirin and other agents that inactivate platelets may prove extremely useful in inhibiting the platelet-neutrophil complexes that collect in the lungs [49]. As the platelet-neutrophil aggregates promote further tissue damage and sequestration of immune cells to the lungs, this could be useful in preventing the hyperimmune response and immunothrombosis. However, antiplatelet drugs combined with LMWH or with unfractionated heparin significantly increases the chance of bleeding, thus making this treatment choice less than ideal, as most patients hospitalized with COVID-19 need to be administered heparin, as noted above [28].

Nafamostat mesylate

Nafamostat mesylate targets the entry of SARS-CoV-2 into cells. As introduced previously, SARS-CoV-2 enters the cell when its spike protein binds to ACE2 and is cleaved by TMPRSS2, allowing the viral envelope to fuse to the plasma membrane and facilitate cell entry. Nafamostat mesylate inhibits TMPRSS2, making it an excellent drug for further testing as a means of preventing SARS-CoV-2 from entering human cells, thereby preventing infection and subsequent CAC [20]. The ability of plasmin to cleave the SARS-CoV-2 spike protein can facilitate viral entry and escalate its pathogenicity. Nafamostat mesylate also shows antiplasmin activity, which could serve to decrease infectivity [14]. However, one should consider how the drug's antiplasmin activity could affect fibrinolysis. Derivatives of this drug could be developed that optimally retain the ability to cleave TMPRSS2 but not the viral spike protein.

Anti-SARS-CoV-2 monoclonal antibodies

Several formulations of human anti-SARS-CoV 2 monoclonal antibodies have been issued EUAs by the FDA for treatment of mild-to-moderate COVID-19 in outpatient adults and children over 12-years-old weighing at least 88 pounds with positive SARS-CoV-2 test and high risk of progression of severe COVID-19, including hospitalization and death [50–53]. These include Regeneron's REGEN-CoV™ (600 mg each of imdevimab and casirivimab), which targets two different regions (epitopes) in the SARS-CoV-2 spike protein [54, 55]; Eli Lilly's bamlanivimab and etesevimab, which bind to overlapping epitopes in the receptor binding domain of the SARS-CoV-2 spike protein [56–58]; and GlaxoSmithKline's (GSK's) sotrovimab (VIR-7831), which binds to a highly conserved region of SARS-CoV-2 spike protein that is shared by SARS-CoV-1 [59, 60].

There are currently no available data indicating superior benefits of any one of these monoclonal antibody formulations over the others. However, as the number of recognized variants of SARS-CoV-2 increases, some monoclonal antibody therapies have been observed to bind the variant virus less well. For example, several variants are resistant to bamlanivimab when it is administered alone, leading the FDA to revoke its EUA for use alone [61]. Due to the prevalence of the Omicron (B.1.1.529) variant, the FDA-authorized use of monoclonal antibody formulations either shown or predicted to be unable to bind to Omicron (including bamlanivimab and etesevimab administered together and the REGEN-COV formulation of casirivimab and imdevimab) has been limited to patients who are likely to have been infected by a variant that remains susceptible to these treatments [62]. A new monoclonal antibody, bebtelovimab (LY3853113; Eli Lilly and Co.), that retains the ability to bind the spike proteins of Omicron and the omicron subvariant BA.2 has been issued an EUA for treatment of mild to moderate COVID-19, either when used alone or in combination with bamlanivimab and etesevimab [58, 63]. As new variants prevail, the list of monoclonal antibodies whose use warrant either EUA or eventual FDA approval will continue to expand or contract accordingly.

These therapies are offered as an intravenous infusion that must be administered in a clinic or hospital setting as soon as possible after diagnosis or within 10 days of the onset of symptoms [50–52]. However, unlike vaccination, the pre-fabricated antibodies administered to patients represent a form of passive immunity that is transient and therefore unlikely to remain in the body longer than a few weeks [64]. In general, monoclonal antibody treatment may worsen the clinical outcomes for hospitalized patients requiring mechanical ventilation or high flow oxygen[63].

The FDA also issued an EUA for a different formulation of monoclonal antibodies for use as a preventive measure for uninfected persons (adults and children over 12 years of age) who are immunocompromised and who have had no recent exposure to an infected person or for whom vaccination is contraindicated [65, 66]. This formulation contains tixagevimab and cilgavimab (marketed by AstraZeneca as Evusheld™), which are neutralizing anti-spike protein antibodies that can block viral attachment to the ACE-2 receptor, thereby preventing SARS-CoV-2 infection [65, 66]. Individuals who have received a COVID-19 vaccine must wait at least two weeks before receiving this formulation. As is the case for monoclonal antibody treatments, the formulation used for pre-exposure prophylaxis provides short term protection and is not intended to replace vaccination in individuals who are eligible to be vaccinated.

Antiviral drugs

Considerable time and effort have been devoted to the development of antiviral drugs specifically inhibit the infectivity or molecular processes of SARS-CoV-2, the most promising of which are remdesivir, molnupiravir, and nirmatrelvir plus ritonavir, as described below.

Remdesivir Remdesivir (GS-5734; marketed by Gilead as Veklury®) was the first antiviral drug approved by the Food and Drug Administration (FDA) for the treatment of COVID-19 [67]. Remdesivir is an inhibitor of the RNA-dependent RNA polymerase (RdRp) that induces stalling during RNA synthesis, leading to an inability of the virus to amplify itself, and is effective in vitro against SARS-CoV-2, SARS-CoV-1, and Middle Eastern respiratory syndrome coronavirus (MERS-CoV) [68, 69]. In a randomized phase III clinical trial, 1,062 patients who were hospitalized with lower respiratory infections that were attributed to COVID-19 were randomly assigned to remdesivir ($n=541$) or placebo ($n=521$) groups. Those treated with remdesivir recovered significantly faster, with a median recovery time of 10 days, while those administered placebo recovered within an average of 15 days ($p<0.001$). Mortality was also reduced on remdesivir, with 6.7% mortality on day 15 and 11.4% by day 20, compared with 11.9% by day 15 and 15.2% by day 29 for patients treated with the placebo. Serious adverse events were reported for 24.6% (131/532) of the patients treated with remdesivir, compared to 31.6% (163/516) of patients in the placebo group. Remdesivir is FDA-approved for treatment of COVID-19 in hospitalized adults and children (≥ 12-years-old, weighing ≥ 40 kg) and available under emergency use authorization (EUA) for hospitalized pediatric patients who weigh at least 3.5 kg [70]. Its intravenous route of administration (100 mg injection) requires that remdesivir is administered in a hospital setting [70].

Molnupiravir Molnupiravir (invented at Drug Innovations at Emory (DRIVE), LLC as EIDD-2801 and developed by Merck and Ridgeback Biotherapeutics as MK-4482) is a ribonucleoside analog that is incorporated by the SARS-CoV-2 RdRp into viral RNA, where it causes mutagenesis [71]. Molnupiravir was authorized in the UK for use to treat hospitalized COVID-19 patients [72] and recommended by the FDA's external advisory committee in November 2021 [73]. In December 2021, the FDA issued an EUA for the treatment of adults who have tested positive for SARS-CoV-2, who are at risk of progression to severe COVID-19 resulting in hospitalization or death, and for whom other FDA-approved treatments are not clinically appropriate [74].

The reason for this seemingly narrow scope of use is that initial excitement for molnupiravir based on preliminary reports from the MOVe-OUT clinical trial that it decreased the rate of hospitalization by 50% was moderated by clinical trial data showing that the final decrease in hospitalizations was only 30% [73]. In this trial, 1,433 participants were enrolled, with 709 being randomly assigned to the molnupiravir group and 699 to the placebo group. Molnupiravir treatment reduced the risk of hospitalization or death to 6.8%, compared to 9.7% for the placebo group. This represents a relative risk reduction of 30% and an absolute risk reduction of 3% (nominal $p=0.0218$). One death was reported for the molnupiravir group, compared to nine for the placebo group. Its oral route of administration would allow treatment of COVID-19 patients outside of the hospital setting, if it is administered as soon as possible after diagnosis or within five days of symptom onset [74].

Paxlovid Paxlovid™ combines Pfizer's new antiviral drug nirmatrelvir (PF-07321332) with existing antiviral ritonavir for treatment of non-hospitalized adults with COVID-19. Nirmatrelvir inhibits the viral 3CL protease required for viral replication, while a low dose of ritonavir is administered simultaneously to slow the metabolism of nirmatrelvir, thereby lengthening its half-life [75]. Since the target of nirmatrelvir is the protease rather than the spike protein, its activity is undiminished in vitro against all previously identified variants of concern, including alpha, beta, delta, gamma, lambda, mu, and omicron. The FDA issued an EUA for the use of Paxlovid™ in December 2021 for use in persons (adults and children over 12-years-old and/or 88 pounds) who have tested positive for SARS-CoV-2 and are at risk of developing severe COVID-19, leading to hospitalization or death [76]. Final data from a clinical trial for high-risk adult patients (evaluation of protease inhibition for COVID-19 (EPIC)-HR; $n=2246$) show Paxlovid™ reduced the risk of hospitalization or death by 89% if taken within three days of symptom onset and by 88% if taken within five days, compared to placebo ($p<0.0001$) [75]. No deaths were reported with Paxlovid™ treatment, while 12 patients (1.2%) who received the placebo died. Interim results for a second trial for standard-risk adults (EPIC-SR) showed that Paxlovid™ reduced the risk of hospitalization by 70% and resulted in no deaths. EPIC-SR evaluated both unvaccinated adults at standard risk (low risk of hospitalization or death) and vaccinated adults with one or more risk factors for severe disease. However, the goal of alleviating all symptoms for four consecutive days remains elusive and the trial continues. Both trials report a decrease in viral load of approximately 10% after five days of treatment [75]. As discussed for molnupiravir, the oral route of Paxlovid™ administration (2×150 mg tablets nirmatrelvir and 1×100 mg ritonavir tablet, twice daily for five days) will allow treatment of COVID-19 patients outside of the hospital setting [75]. It should be administered as soon as possible

after a positive test for SARS-CoV-2 (diagnosis of COVID-19) or within five days of the onset of symptoms [76].

Comprehensive approach

Holistically, Berkman and Tapson (2020) evaluated multifaceted therapeutic options for CAC [42]. These authors point out that, strictly from the viewpoint of thrombosis, a rational treatment option for the most critically ill patients might theoretically include a full-dose anti-coagulant to treat the thrombotic storm in combination with corticosteroids and/or interleukin-6 antagonists to treat or prevent a cytokine storm but warn that this recommendation is based on weak evidence [42]. These findings are consistent with those of other researchers. Unfortunately, despite more than two years having passed since the first reported SARS-CoV-2 infection, there is no single therapeutic approach to treat COVID-19. Due to the complexity of the disease, the variety of effects it has on the body systems, and the variability of CAC among every patient, no comprehensive treatment regimen provides the gold standard of care applicable to every patient.

In addition to the specific treatment(s) needed to fight COVID-19 in general, Asakura and Okagawa (2021) contend there are certain periods during this viral infection that drugs of differing functions should be administered [14]. They propose that at the beginning of treatment, patients should be treated with antiviral and antithrombotic therapies. Without specifying a length of time, they propose that in the middle of the infection, healthcare providers should continue antithrombotic therapy, slowly wean patients from antiviral therapy, and begin to administer an anti-cytokine therapy that would optimally continue until the end of severe signs and symptoms. Broadly, these investigators believe that a combination therapy administered with this general timeline would provide the best patient outcome [14].

Summary and conclusions

COVID-19-associated coagulopathy (CAC) has proven to be a severe, distinctive, and multifaceted coagulation response to SARS-CoV-2 infection that causes fatal macrovascular and microvascular thromboses, organ dysfunction, and death. The mechanisms underlying CAC include dysregulation of the coagulation system, endothelial damage, hyperinflammation, and immunothrombosis. Monitoring of CAC is complicated, as only two laboratory values are widely considered useful, and the extent of the coagulopathy is often not determined until autopsy. Because of this, it is crucial to continue research to find reliable and specific biomarkers to aid healthcare providers in understanding the severity of a patient's CAC before the patient succumbs to it. D-dimer and fibrinogen are currently the most relied-upon markers of CAC, as they show direct correlation with severity, but they alone are not wholly representative of the disease state. As CAC causes macrovascular and microvascular thromboses, heparin is the antithrombotic drug of choice. However, since it does not prevent many of the vascular coagulation incidents and can cause bleeding, investigators still must identify an antithrombotic agent that can sufficiently prevent the widespread clotting while not putting the patient at risk of severe bleeding. Furthermore, although tocilizumab is an excellent drug for those experiencing hypercoagulability as the result of hyperinflammation and cytokine storms, an anti-IL-6 drug would not be useful for someone being treated with ECMO who needs an antithrombotic agent that can target hypercoagulability caused by extensive tissue damage. Also, nafamostat mesylate is a promising drug that can inhibit SARS-CoV-2 from entering human cells, but once a patient shows symptoms of COVID-19, the virus will have been locally established in the body. If the virus has already reached the lungs and caused enough damage to induce CAC, administration of nafamostat mesylate may be less useful, especially because it also acts as an anti-plasmin drug and could potentially worsen CAC. Investigators must continue to search for ways to prevent CAC, determine the severity with the best biomarker, and treat CAC swiftly and adequately so that patients will no longer die from this lethal complication of COVID-19.

Future research

Identification of a biomarker that directly indicates the amount of endothelial damage occurring in the lungs and throughout the body would be very beneficial for monitoring and managing CAC. Ang-2 is a biomarker that warrants further study, as its release from endothelial cells when they are damaged either by SARS-CoV-2 entry or during a clotting event can signify the extent of endothelial damage. Since not every hospital may possess the ability to quickly measure levels of Ang-2 in patients, more studies on the usefulness of this protein and its relationship to CAC would be valuable to determine whether its monitoring would improve the outcome of COVID-19 patients.

Furthermore, the studies that indicate the higher percentages of VTEs have one thing in common, i.e., the use of prophylactic point-of-care monitoring to determine whether patients are experiencing VTEs in real time. This finding could be incredibly useful for the future of COVID-19 patient care because equipping hospitals with more ultrasound machines for screening patients for VTEs would mean that VTEs could be detected before they travel to the lungs and cause complications or death. Unfortunately, in a pandemic, providing patients with such a service could prove difficult with both hospital staff and

resources stretched thin. However, at a time where any prophylactic measure that could lead to decreased mortality is desperately needed, implementation of daily bedside ultrasound monitoring of the legs of COVID-19 patients should be seriously considered by hospitals.

Additional future studies will be needed to determine whether nafamostat mesylate could be beneficial if given prophylactically to people who have been exposed to COVID-19. Since it is considered effective in preventing the entrance of the virus into the endothelial cells in the lungs, it would be most helpful in patients who do not yet have COVID-19 but who are at risk of contracting it. Moreover, the antifibrinolytic effect of nafamostat mesylate needs to be studied in hospitalized COVID-19 patients. Not only could it propagate CAC, but it could also reduce the usefulness of D-dimers in monitoring the status of CAC in a patient, since D-dimers are only formed during fibrinolysis. If fibrinolysis were to be reduced or inhibited by nafamostat mesylate and a healthcare provider were to neglect to consider that D-dimer levels would no longer reflect the extent of clotting, the risk of thrombosis may be wrongfully assessed. This could result in failure to detect and treat CAC. The use of drugs that can treat one mechanism or facet of CAC but that put patients at risk of another mechanism or aspect of CAC is a dangerous game of cause and effect. Focus should be placed on finding a specific treatment for CAC that does not in turn cause another risk to the patient, if such a treatment should be found.

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Declarations

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